Supplemental Information

Supplemental Tables

Table S1. Clinicopathologic features of patient samples

Category	Sample # (32 Total)
Age of diagnosis	
≤65	20
>65	12
Gender	
Male	12
Female	20
Smoking History	
Current	7
Reformed	20
Never	5
Pack years	
≤20	8
>20	24
Tumor Stage	
I	6
II	3
III	7
IV	8
Normal Lung	8

Table S2. Clinicopathologic features of matched stage I samples

Category	Sample # (11 Total)
Age of diagnosis	
≤65	5
>65	6
Gender	
Male	6
Female	5
Smoking History	
Current	2
Reformed	9
Never	0
Pack years	
≤20	0
>20	11

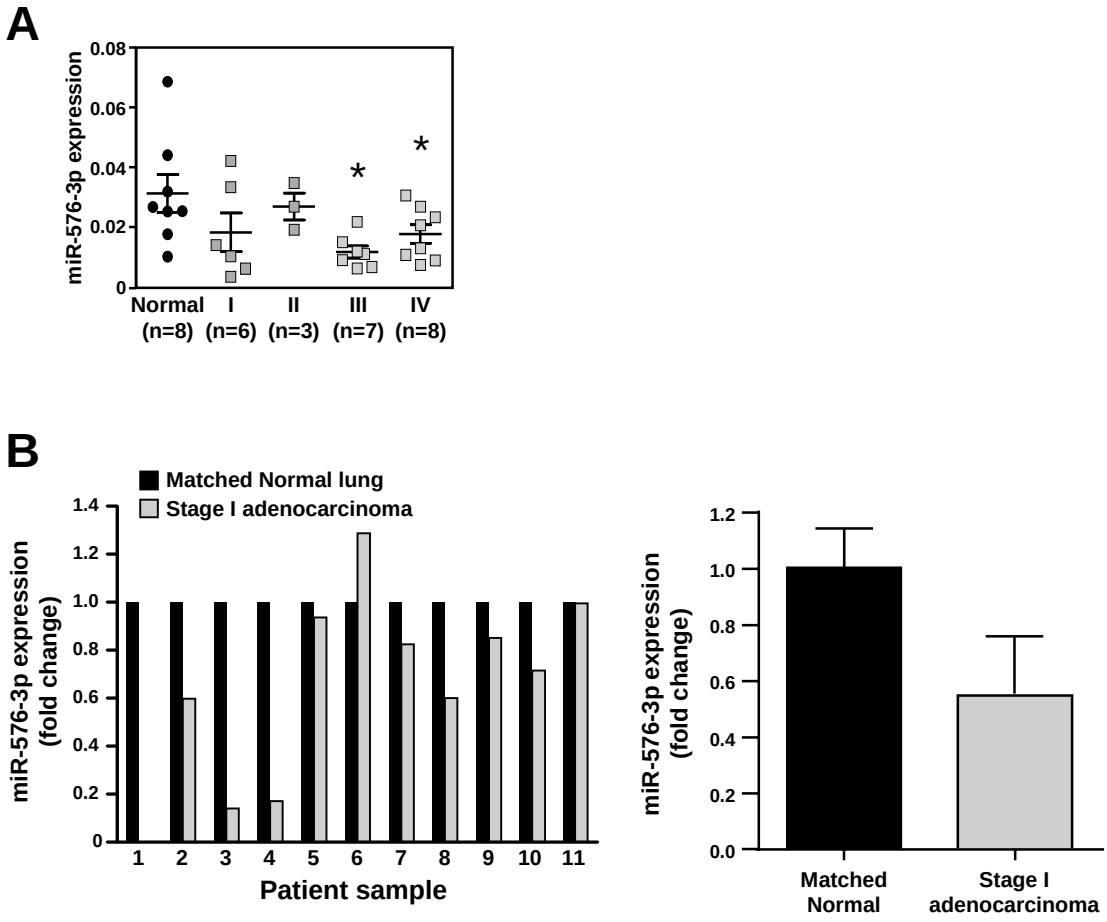
Supplemental Figure Legends

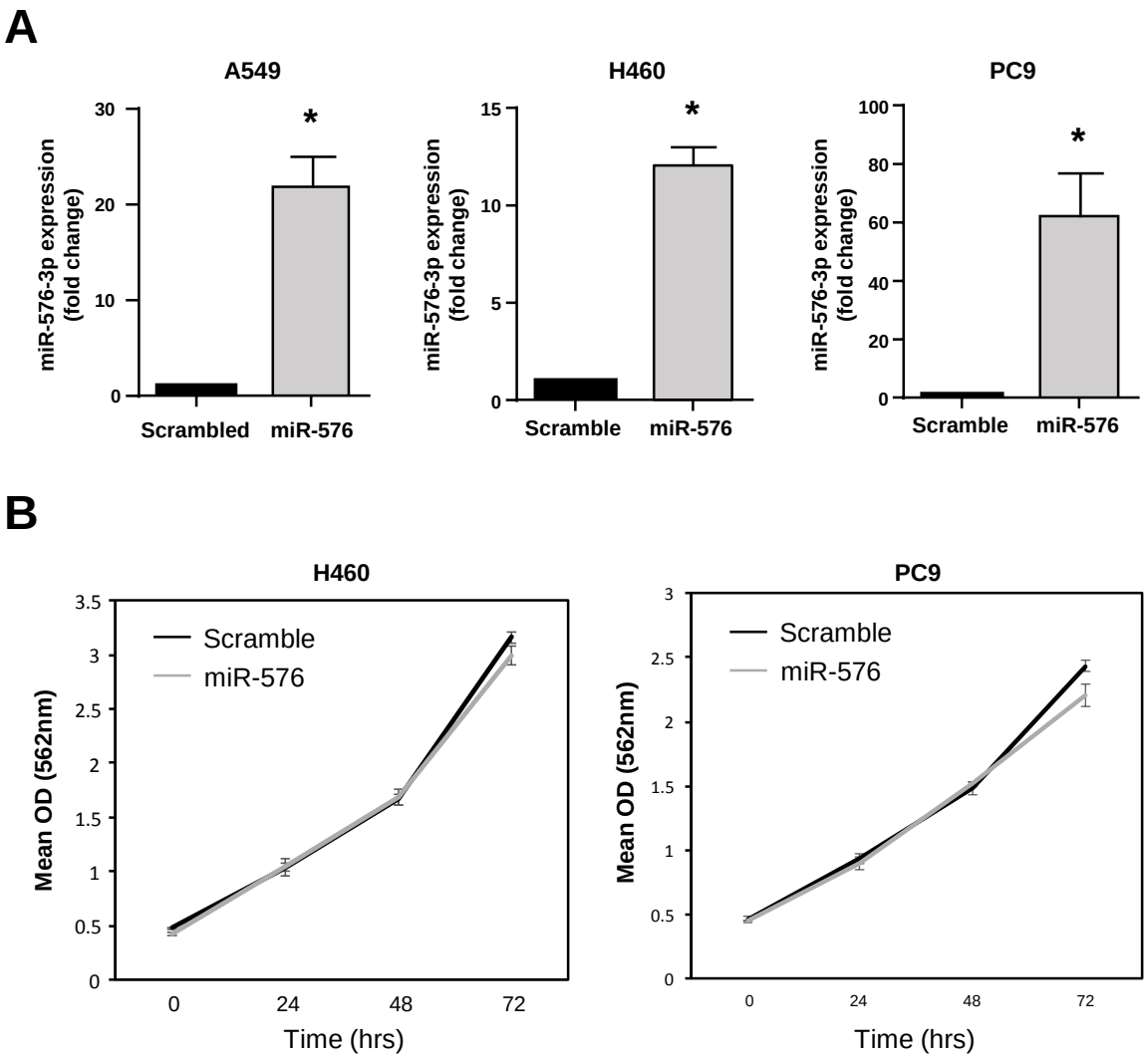
Supplemental Figure 1. miR-576-3p expression is downregulated in late stage lung adenocarcinoma. A) qRT-PCR data (triplicates) from figure 1B separated into individual stages of lung adenocarcinoma or normal lung controls; Stage III, *P=8.3x10^{-3}; Stage IV, *P=0.038, one-tailed t-test. All data are relative to the endogenous control RNU6B small RNA. B) qPCR array was previously performed on lung adenocarcinoma patient samples for which there was matched normal adjacent lung[14]. miR-576-3p expression in each normal tissue was normalized to 1 and fold change determined for its matched tumor sample (left). The mean of the fold changes of all the patient samples is shown on the right; SEM; P=0.086, two-tailed t-test.

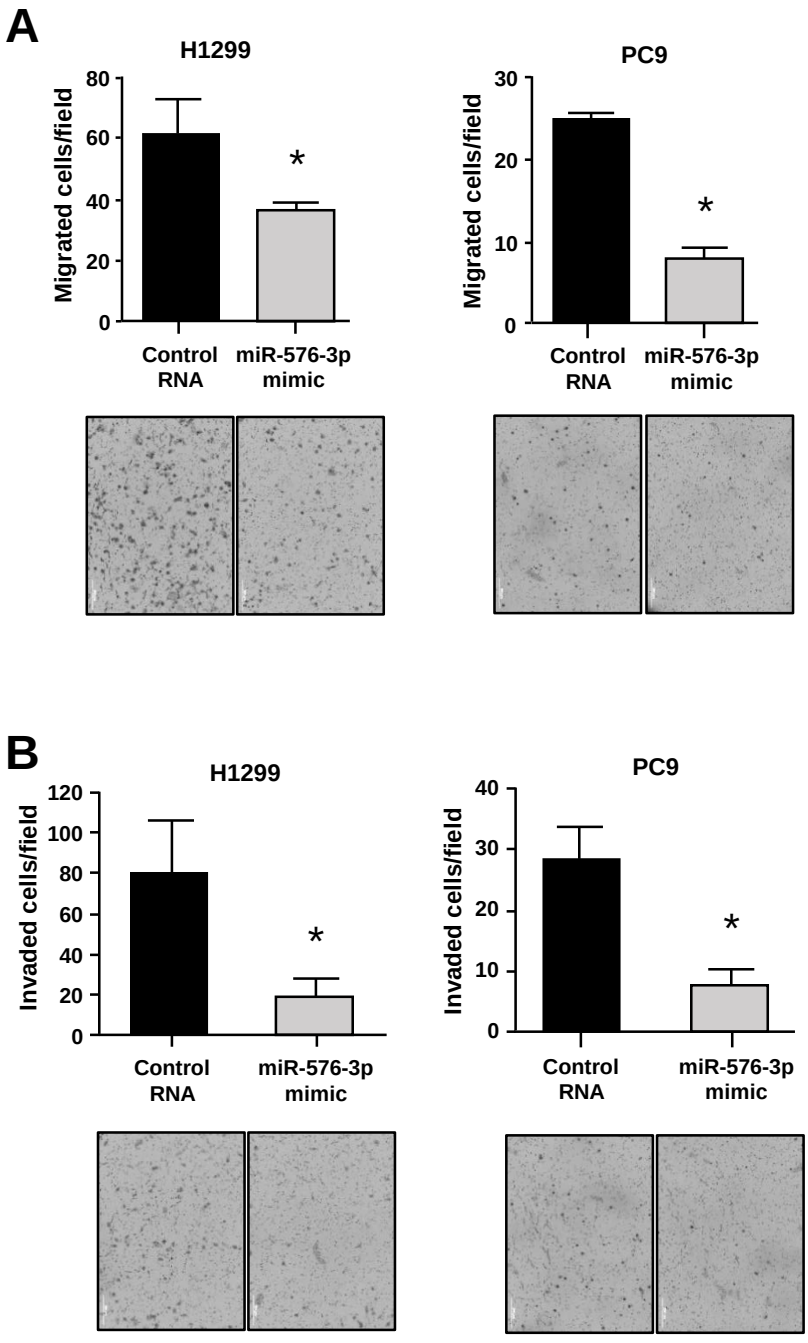
Supplemental Figure 2. miR-576 overexpression in lung adenocarcinoma cells does not affect proliferation. (A) qRT-PCR was performed (triplicates) on the indicated lung adenocarcinoma cells infected with a retroviral vector encoding miR-576 or scrambled RNA. Fold change displayed with the scrambled sample set at 1; SEM; A549 P=2.7x10^{-3}; H460 P=9.4x10^{-7}; PC9 P=3.9x10^{-5}, two-tailed t-test. (B) MTT assays (quadruplicate) were performed at the indicated intervals. Data are representative of two independent experiments, SEM.

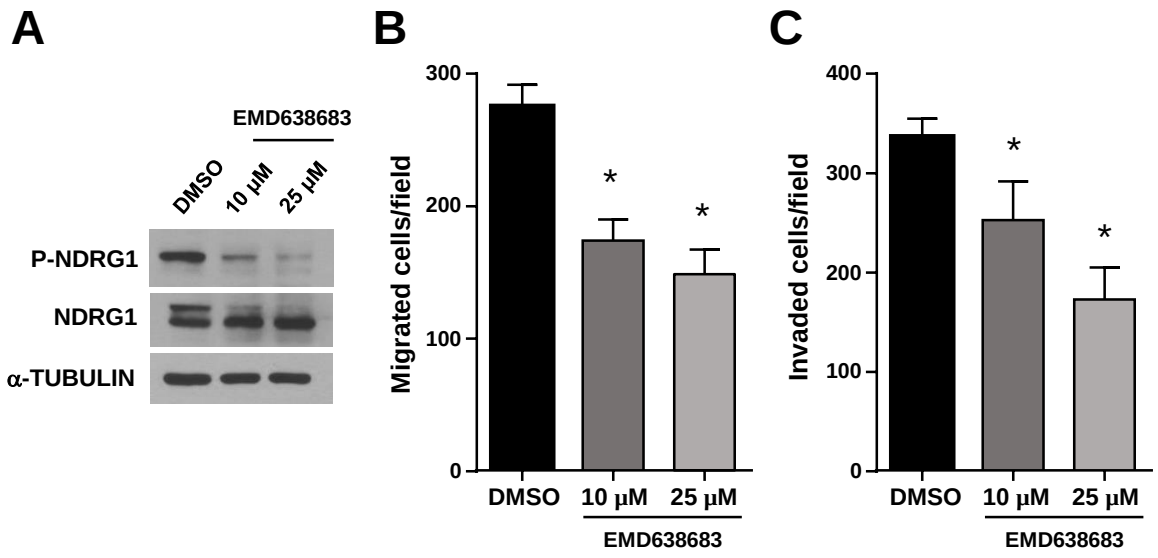
Supplemental Figure 3. miR-576-3p inhibits migration and invasion of lung adenocarcinoma cells. Migration (A) and invasion (B) assays (triplicates for both) of lung adenocarcinoma cells transfected with miR-576-3p mimic or RNA control. Data are representative of two independent experiments; SEM; A, H1299, *P=7.6x10^{-3}; A, PC9, *P=1.1x10^{-4}; B, H1299, *P=0.045; B, PC9, *P=0.012, two-tailed t-test. Representative images shown (10x).

Supplemental Figure 4. Pharmacological SGK inhibition prevents downstream target phosphorylation and cell migration and invasion. H1299 cells were treated with vehicle control (DMSO) or with EMD638683 at the concentrations indicated. Western blots (A) and transwell migration (B) and invasion (C) assays performed; SEM; C, 10μM **P*=1.3x10^{-4}, 25μM **P*=5.2x10^{-5}; D, 10μM **P*=8.4x10^{-3}; D, 25μM **P*=1.2x10^{-4}, two-tailed t-test.


A
miR-576-3p expression
0.08
0.06
0.04
0.02
0
Normal (n=8)
I (n=6)
II (n=3)
III (n=7)
IV (n=8)
*
*
B
Matched Normal lung
Stage I adenocarcinoma
miR-576-3p expression (fold change)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11
Patient sample
miR-576-3p expression (fold change)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Matched Normal
Stage I adenocarcinoma


A
A549
H460
PC9
miR-576-3p expression (fold change)
30
20
10
0
15
5
100
80
60
40
20
*
Scrambled
miR-576
Scramble
B
H460
PC9
Mean OD (562nm)
3.5
3
2.5
2
1.5
1
0.5
0
Scramble
miR-576
0
24
48
72
Time (hrs)


A
H1299
Migrated cells/field
80
60
40
20
0
*
Control RNA
miR-576-3p mimic
PC9
Migrated cells/field
30
20
10
0
*
Control RNA
miR-576-3p mimic
B
H1299
Invaded cells/field
120
100
80
60
40
20
0
*
Control RNA
miR-576-3p mimic
PC9
Invaded cells/field
40
30
20
10
0
*
Control RNA
miR-576-3p mimic


A
EMD638683
DMSO
10 μM
25 μM
P-NDRG1
NDRG1
α-TUBULIN
B
Migrated cells/field
300
200
100
0
*
*
DMSO
10 μM
25 μM
EMD638683
C
Invaded cells/field
400
300
200
100
0
*
*
DMSO
10 μM
25 μM
EMD638683